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PHYCOCHEMICAL EXAMINATION OF *PADINA TETRASTROMATICA* (DICTYOTALES, PHAEOPHYTA)

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ABSTRACT

A marine brown alga, Padina tetrastromatica Hauck, collected from Buleji near Karachi, has been investigated phycochemically. Five saturated fatty acids, i.e. isomyristic, palmitic, margaric, stearic and arachidic, three unsaturated fatty acids, i.e. palmitoleic, oleic and tetradecatrienoic have been detected and identified by preparation of their methyl esters and GC-MS analysis. Two sterols, i.e. 24-methyl cholesterol [1] and 24-methylene cholesterol [2] have been isolated and identified by GC-MS, El and NMR, which are being reported for the first time from the genus *Padina*

Introduction

Phycochemistry is the study of the natural products and the chemical constituents occurring in algae, from a biological point of view (Shameel, 1990a). *Padina tetrastromatica* Hauck is a striped, yellowish brown, fan shaped alga, which becomes olive green on drying. Its [halt are irregularly cleft into narrow lobes, having involuted apical margin (Misra, 1966). It grows in shallow, sand covered rocky pools of mid- and lower littoral at Karachi and the adjacent coastal areas of Pakistan (Shameel, 19906). Although, other species *of Padina* have been investigated chemically in detail (e.g. Percival and Mian, 1972; Iatrides *et al.,* 1983; Parekh *et al.,* 1984; Combaut *et al.,* 1985; Khafaji, 1986), but there are a few chemical studies made on *P.* tetrastromatica (Dhargalkar *et* at, 1980; Rao and Pullaiah, 1982). Even from the coast of Karachi *P. pavonia (L.)* Lamour. has been phycochemically examined (Zahid *et al.,* 1983; Qasim, 1986), but no such study has ever been made on *P. tetrastromatica.* Therefore in the present study an attempt was made to isolate and characterize the fatty acids and sterols present in this seaweed.

Materials and Methods

Padina tetrastromatica was collected from mid-littoral zone at the coast of Buleji, near Karachi during October 1986. The visible epiphytes and animal castings were washed out with running water.

Isolation of fatty acids:

About 1.5 kg of the thalli were stored in deep freezer and later soaked in $CHCl_3$ and hexane (1:1 v/v) for 20 days. After percolation the organic layer was evaporated under reduced pressure and a residue of 13.39 g was obtained. The residue was saponified with ethanolic potash (10% KOH in 50% ethanol) under reflux for 8 hours at 100°C. The mixture was concentrated under reduced pressure and thereafter water and Et_2O were added and extraction with Et_2O was repeated 3 times. The aqueous alkaline fraction was acidified with 6N HCl (pH 5-6) and then extracted several times with Et_2O. The total Et_2O fraction was dried over anhydrous Na_2SO_4 and on evaporation of Et_2O a dark brownish residue (4 g) was obtained.

The fatty acid fraction weighing about 0.5 mg was dissolved in MeOH and 05 ml of diazomethane was added. The reaction mixture was kept over night at room temperature (28 °C) and was evaporated under reduced pressure. The aliquotes were directly injected into GC-MS. The GC-MS of fatty acid methyl esters was performed on a Hewlett Packard GC with a 11373 DEC computer data system and a 1.2 m x 4 mm packed glass capillary column coated with gas chrome Q (100-120 mesh, OV 101, 1%). The column temperature was programmed between 70° -250°C with a rate of increase of 8°C per minute. The carrier gas (He) flow rate was 32 ml/min and injector temperature was 250°C.

Isolation of sterols:

The healthy specimens (539 kg) were dried under shade. The dried algae (1.55 kg) were homogenized and extracted under soxhlet with MeOH for 6 hours. On evaporation of organic solvents the syrupy residue weighed 25 g. This material was partitioned between water and EtOAc. The EtOAc fraction on evaporation under vacuum was found to be 73 g and this sterol containing fraction was subjected to silica gel column chromatography. The fractions eluted with hexane-diethyl ether (90:10) yielded a crystalline compound characterized as hexadecanoic acid. After increasing the polarity of the solvent, hexane-diethyl ether (75:25) afforded a mixture of sterols.

The sterols were purified through preparative thin layer chromatography (TLC) with hexane-diethyl ether-acetic acid (1:1:0.08 v/v/v). It furnished two spots having R_f values 0.93 and 0.82 with Lieberman-Buchard reagent. Both the compounds were recrystallized with CHCB and subjected to mass and NMR spectral scanning.

Results and Discussion

The entire thalh of *P. tetrasaomatica* were analysed for their fatty acid composition by using GC-MS technique, as this procedure is more simple and accurate than other traditional methods. The results obtained have been presented in Table-1. It indicated the presence of five saturated fatty acids, Le. methyl isomyristate (C15:0), methyl pahnitate (C17:0), methyl margarate (C18:0), methyl stearate (C19:0) and methyl arachidate (021:0) and three unsaturated fatty adds, i.e. methyl pahnitoleate (C17:1), methyl oleate (C19:1) and methyl tetradecatrienoate (C153).

Table-1

Fatty acids of *Padina tetrastromatica* analysed as methyl esters.

Systematic Name	Common Name	Molecular Formula	Mol. Wt.	Ret.Time (Min.)	Rel. % age	Mass and Fragmentation Pattern
A. Saturated fatty acid methyl esters						
Methyl tetradecanoate	Methyl isomyristate	$C_{15}H_{30}O_2$	242	17'20"	6.0	GC-MS m/z 242 (M^+, $C_{15}H_{30}O_2$), 211 (M^+ -31), 199 (M^+ -43), 185, 178, 157, 143, 129, 111, 87, 74 (100%).
Methyl hexadecanoate	Methyl palmitate	$C_{17}H_{34}O_2$	270	22'24"	36.6	GC-MS m/z 270 (M^+, $C_{17}H_{34}O_2$), 239 (M^+ -31), 227 (M^+ -43), 213, 199, 185, 171, 157, 143, 129, 115, 101, 87, 74 (100%).
Methyl heptadecanoate	Methyl margarate	$C_{18}H_{36}O_2$	284	24'44"	3.4	GC-MS m/z 284 (M^+, $C_{18}H_{36}O_2$), 253 (M^+ -31), 241 (M^+ -43), 199, 185, 171, 143, 129, 115, 101, 87, 74 (100%).

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Systematic Name	Common Name	Molecular Formula	Mol. Wt.	Ret.Time (Min.)	Rel. % age	Mass and Fragmentation Pattern
Methyl octadeca-noate	Methyl stearate	$C_{19}H_{38}O_2$	298	27'04"	13.6	GC-MS m/z 298 (M^+, $C_{19}H_{38}O_2$), 267 (M^+ -31), 255 (M^+ -43), 241, 213, 199, 185, 171, 157, 143, 129, 115, 101, 87, 74 (100%)
Methyl eicosanoate	Methyl arachi-date	$C_{21}H_{42}O_2$	326	31'30"	3.8	GC-MS m/z 326 (M^+, $C_{21}H_{42}O$), 283 (M^+ -43), 256, 239, 207, 171 (M^+ -155), 157 (M -169), 143 (M^+ -183), 111, 101 (M^+ -255), 99, 87, 74 (100%).
B. Unsaturated fatty acid methyl esters:						
Methyl hexadece-noate	Methyl palmito-leate	$C_{17}H_{32}O_2$	268	21'42"	1.9	GC-MS m/z 268 (M^+, $C_{17}H_{32}O_2$), 236 (M^+ -32), 194 (M^+ -74), 152, 123, 111, 97, 83, 74 (100%).
Methyl octadece-noate	Methyl oleate	$C_{19}H_{36}O_2$	296	26'22"	22.2	GC-MS m/z 296 (M^+, $C_{19}H_{36}O_2$), 264 (M^+ -32), 222 (M^+ -74), 180, 123, 111, 97, 83, 74 (100%).
Methyl 2,4,5-tet-radecatri-enoate	Methyl tetrade-catri-enoate	$C_{15}H_{24}O_2$	236	10'02"	3.8	GC-MS m/z 236 (M^+, $C_{15}H_{24}O_2$), 205 (M^+ -31), 193 (M^+ -43), 180, 165, 147, 137, 123, 109, 81, 67, 57 (100%).

The analysis of fatty acids revealed that no acid lower than C15 was present. This indicates maturity of the material by virtue of the fact that during development the fatty acids with lower length of carbon chain disappear (Breuer et al, 1987). A similar observation was also made on other brown algae, *Stoechospennum marginatum* (C. Ag.)

Katz. (Shaikh *et al*, 1990) and *Colpomenio* sinuoso (Roth) Derbrs et Solier (Shaikh *et al.*, 1991). The unsaturated fatty acids were lesser in number as well as quantity (28%) than saturated ones (63.4%). Probably they are dominated by the formation of saturated fatly acids during the building up of food storage process. Similar observations were made on other seaweeds collected from Karachi (Qasim, 1986; Shamed, 1990a).

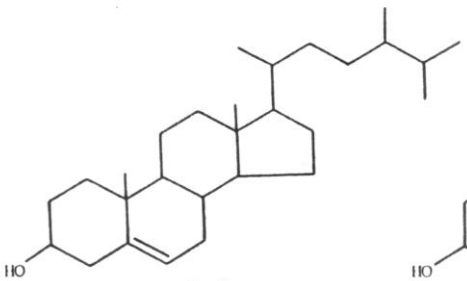
Methyl palmitate was found to be present in the largest quantity (36.6%) in *P. tetrastronratica*. Palmitic acid has also been observed as the major fatty acid in *P. gynlnospora* (Kütz.) Sond. (Parekh *et al.*, 1984) and *P. pavonia* (Qasim, 1986). Among unsaturated fatty acid methyl esters, methyl oleate was present in the largest quantity (22.2%) in *P. tetrastrornatica.* Oleic acid has also been observed as the major unsaturated fatty acid in other brown algae (Shamed, 1990a). Methyl tetradecatrienoate, a triunsaturated fatty acid, occurred in small quantity (3.8%). It has also been detected in small quantity as 3.80% in *Stoechospennum marginatum* (Shaikh *et al*, 1990) and as 2.83% in *Colpomenia sinuoso* (Shaikh *et al.*, 1991).

The sterol fractions of *P. tetrastromalica* were purified by preparative TLC and detected through Liebermann-Ruchardt test. The isolated pure sterols *were* subjected to analysis by mass spectrometry and the following spectral data have been obtained.

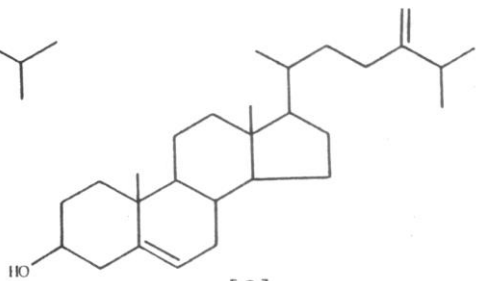
24-Methyl cholesterol [1]: Mass (El) m/z 400 (M^+, C28114aO, 60%), 385 (M^+ - CH_3, 4%), 382 (M^+-H_2O, 19%), 367 (M^+-CH_2-H_2O, 2%), 315 (86%), 300 (86%), 273 (M^+ -side chain, 7%), 271 *(34%), 255* (48%), 231 (43%), 207 (15%), 107 (11%).

24-Methylene cholesterol [2]: Mass (EI) m/z 398 (M^+, $C_{28}H_{46}O$, 10%), 384 (M^+ - CH_2, 30%), 368 (M^+-$2CH_3$, 18%), 314 (M^+ -C_{24} (28) double bond RDA fragment, 26%), 271 (18%), 255 (16%), 213 (24%), 171 (12%), 145 (34%), 81 (76%).

^{1}H-NMR (400 MHz, $CDCl_3$), ppm. 0.69 (H-18), 1.01 (H-19), 1.03 (d, J = 6.5 Hz, H-21), 0.90 (d, 1 = 6.5 Hz, H-26 and H-27), 3.5 (m, H-3), 5.30 (distort t, H-6), 4.60 and 4.70 (m, H-28).



[1]



[2]

The data indicated the presence of 24-R-methyl-cholest-5-en-3B-ol, *Le.* 24-methyl cholesterol [1] and 24-R-methylene-cbolest-5-en-38-ol, *i.e.* 24-methylene cholesterol]2]. The former was confirmed by comparison with an authentic sample isolated previously from *Iyengana stellata* (BΦrg.) Borg. (Usmanghani *et al,* 1987) and the latter by the details of its ^{1}H-NMR spectra.

Cholesterol and fucosterol are the only two sterols reported so far from various species of *Padina, e.g. P. pavonia* (Iatrides *et al*., 1985), *P. tetras*r*omatica* (Rao and Pullaiah, 1982) and *P. vickersfae* Hoyt in Howe (Combaut *et d.,* 1985). However, this is the first report about the occurrence of 24-methyl cholesterol [1] and 24-methylene cholesterol (2] from any species of *Padina.* Although, 24-methylene cholesterol was found to be present in *several* brown seaweeds, *e.g. Endarachne binghwniae* J. Ag. (Bano *et al.,* 1987) and *Laminatia digitata* (Patterson, 1971), but 24-methyl cholesterol has so far been reported only from a brown alga, *Iyengaria stellam* (Usmanghani *et al,* 1987).

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References

Bano, S., Perveen, S., Ahmad, V.U., Shafiuddin, Bano, N. and Shameel, M. (1987). Chemical constituents of *Endarachne binghamiae* (Scytosiphonales, Phaeophyta) from the Karachi coast. *Bot. Mar.* **30**(5): 371-372.

Breuer, B., Stuhlfauth, T., Pock, H. and Huber, H. (1987). Fatty acids of some Cornaceae, Hydrangiaceae, Aquifoliaceae, Hamamelidaceae and Styraceae. *Phytochem.* **26**: 1441-1445.

Combaut, G., Yacoubou, A., Piovetti, A. and Kornprobst, J. N. (1985). Sterols of the Senegalese brown *alga Pa*d*ina vickersiae. Phytochem.* **24**: 618-619.

Dhargalkar, V. K., Jagtap, T. G. and Untawale, A. G. (1980). Biochemical constituents of seaweeds along the Maharashtra coast. *Ind. I. Mar. Sci*. **9**: 297-299.

latrides, M. C., Artuad, J. and Vicente, N. (1983). Sterol composition of Mediterranean marine plants. *Oceanol. Acta*. **6**: 73-77.

Khafaji, A. K. (1986). Alginate and laminarine composition of some brown algae from Red Sea near Jeddah, Saudi Arabia. *Pak. I. Rot.* **18**(2): 351-353.

Mica, J. N. (1966). Phaeophyceae in India. ICAR, New Delhi, p. 203.

Parekh, K. S., Parekh, H. H. and Rao, P. S. (1984). Fatty acid content of some Indian marine algae. *Ind. J. Mar. Sci*. **13**: 45-46.

Patterson, G. W. (1971). The distribution of sterols in algae. *Lipids.* **6**: 120-127.

Percival, E. and Mimi, A. J. (1972). Fucose-containing polysaccharides in brown seaweeds. *Proc. Int. Seaweed Symp.* **7**: 443-446.

Qasim, R. (1986). Studies on fatty acid composition of eighteen species of seaweeds from

the Karachi coast. *J. Chem. Soc. Pak* **8**(2): 223-230.

Rao, C. B. and Pullaiah, K C. (1982). Chemical examination of marine algae of Visakhapatnam coast: Part II constituents of *Padina* tetrartromafca Hauck. *Ind J. Chem.* **21**B: 605-606.

Shaikh, W., Shamed, M., Hayee-Memon, A., Usmanghani, K., Bano, S. and Ahmad, V. U. (1990). Isolation and characterization of chemical constituents of *Stoechospennum* marginatum (Dictyotales, Phaeophyta) and the[i]r antimicrobial activity. *Pak J. Pharm. Sci*. **3**(2): 1-9.

Shaikh, W., Shamed, M., Ahmad, V. U. and Usmanghani, IC (1991). Phycochemical studies on *Colpomenia sinuosa* (Scytosiphonales, Phaeophyta). *Bot. Mar*. **34**(2): 77-79.

Shameel, M. (1990a). Phycochemical studies on fatty acids from certain seaweeds. *Bat. Mar.* **33**(5): 429-432.

Shameel, M. (1990b). *A p*re*limina*ry *check-list of ma*ri*ne algae f*ro*m the coast and* inshore *wate*rs *of Pakistan.* MAHQ Biol. Res. Cent., Kar. Univ., Karachi, S6 Pp.

Usmanghani, K., Shamed, M., Siddiqui, S. and Alam, M. (1987). Studies on the sterols of a brown seaweed *Iyengaia stellata* from Pakistan. *Pak J. Bot.* **19**(2): 249-252.

Zahid, P. B., Hasni, S. and Bawani, Y. (1983). Taxonomy and some biochemical values of *Ulva* indica and *Padina* pavonia collected from Karachi coast. *J. Pharm. Univ. Kar.* **1**: 155-161.